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Contrasting feeding habits of post-larval and adult *Astyanax* cavefish

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Abstract

The subterranean environment is often described as "extreme" and food poor. Laboratory experiments have shown that blind Mexican tetra *Astyanax mexicanus* (De Filippi, 1853) cavefish are better at finding food in the dark than surface fish. Several morphological and behavioural attributes that could foster this obvious adaptive response to cave environments have been described. Nonetheless, it is currently unknown what young cavefish actually eat in their natural cave environment. Our results from the Pachón cave in México during the dry and rainy season show that fry are efficient predators in their natural cave environment. Their primary food item is aquatic crustaceans. The guts of post-larval, pre-juvenile stage individuals (n=9) contained an average of 17.9 water fleas (Cladocera), copepods, ostracods, and isopods. Thus, the fry in this cave are well-fed. The Pachón cave environment does not appear to be "food poor" for juvenile cavefish. Food regimes change between post-larval and adult stages to become more dependent on partially decomposed material, guano, or detritus from the mud. We discuss the data with regards to our current developmental and genetic understanding of cavefish morphological and behavioural evolution, particularly regarding its enhanced Vibration Attraction Behaviour (VAB).

Keywords

Predation, gut contents, troglomorphy, VAB, neuromast

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Introduction

The blind Mexican tetra *Astyanax mexicanus* (De Filippi, 1853) has become a well-established model system in evolutionary developmental biology (EvoDevo). This species has been the main contributor in the understanding of the genetic and developmental controls of troglomorphic features. There are over 30 known caves harbouring *Astyanax* cavefish populations in México (Mitchell et al. 1977; Espinasa et al. 2001). Their conspecific surface-dwelling morph lives in nearby surface streams throughout most of México. The eyed and pigmented surface morph and the eyeless, depigmented cave morph are inter-fertile, making the species well-suited for experimental manipulations (Jeffery 2012; Elipot et al. 2014).

Literature often states that the cave morph is more efficient at finding food in darkness. Multiple morphological and behavioural attributes have been described to support this statement, such as a higher number of taste buds (Yamamoto et al. 2009; Varatharasan et al. 2009), higher chemosensory capabilities (Protas et al. 2008; Bibliowicz et al. 2013; Hinaux et al. 2016), an enhanced number of cranial neuromasts (Yoshizawa et al. 2012), modulation in early developmental signalling pathways influencing brain development and organization (Yamamoto et al. 2004; Pottin et al. 2011), and a behaviourally more efficient posture with respect to the substrate when bottom feeding (Schemmel 1980).

Increased efficiency in food finding has been supported by five controlled observations or experiments in which cavefish directly outcompeted surface fish for a limited amount of food. Three of these observations were in adult fish (Hüppop 1987; Yoshizawa et al. 2010; Wilkens and Hüppop 1986), and two supported that the enhanced skills were already in place as early as 25 days after birth (Espinasa et al. 2014; Hinaux et al. 2016). For example, Hüppop (1987) showed that under competitive conditions, cavefish found about 80% of all pieces of beef-heart muscle provided in the dark whereas the epigean fish were successful at finding only 20%. This suggests enhanced smell and chemical detection in cavefish. Direct measurements of the size of the olfactory pits have shown that eyeless cavefish have a wider olfactory pit than eyed surface fish. The width of the olfactory pit is on average 12.9% larger due to the eye-dependent developmental processes (Yamamoto et al. 2003; Hinaux et al. 2016). An enlarged olfactory pit could result in an enhanced sense of smell, which could directly correlate with the feeding skills of eyeless fish (Bibliowicz et al. 2013). Indeed, 30 day-old juvenile cavefish were recently shown to have outstanding olfactory capabilities at a magnitude 105 higher than surface fish for food-related amino-acid odorant cues (Hinaux et al. 2016).

In two studies (Yoshizawa 2010; Espinasa et al. 2014), food finding efficiency was tested using competition assays where fish had to capture live *Artemia* larvae (nauplii). The results suggest that both adult and young *Astyanax* have enhanced abilities for prey capture. For example, in the Espinasa et al. (2014) study, 25 day-old cavefish and surface fish raised under light/dark conditions were paired to compete for *Artemia* nauplii in the dark. On average, cavefish captured more *Artemia* than surface fish in each

paired match. Both studies suggest that other complex skills, apart from smell, may be enhanced in cavefish to allow for efficient predation.

Small crustaceans such as copepods disturb the water at 30–40 Hz when swimming (Montgomery and Macdonald 1987). Vibration attraction behaviour (VAB) is the ability of fish to swim toward the source of such vibrations and has been shown to be advantageous for *Artemia* feeding competition assays in the dark by adult cavefish (Yoshizawa et al. 2010). In cavefish, the detection range of the superficial neuromasts in the lateral line system is tuned to 35 Hz (Yoshizawa et al. 2010). The cupulae of cavefish superficial neuromasts are about 300 μm in length compared to about 42 μm in surface fish (Teyke 1990). Neuromasts within the eye orbit and in the suborbital region are about twice as sensitive in young adult cavefish as in surface fish (Yoshizawa et al. 2014). VAB is typically seen in cavefish, but rarely in surface fish. While in the laboratory VAB is not statistically evident before cavefish reach three months of age (Yoshizawa et al. 2010), results by Espinasa et al. (2014) suggest it is possible that some of the physiological bases behind the behaviour are active in young fish.

While it is evident that *Astyanax* have undergone significant modifications in feeding skills, the actual food sources of *Astyanax* remain unclear in their natural cave environment. Some authors have mentioned that their food consists almost completely of bat guano rather than live and mobile organisms (Kasumyan and Marusov 2015; Wilkens 1972; Mitchell et al. 1977; Hüppop 1987). If this was the case, it would be difficult to explain the selective value of VAB found in multiple cave populations. The purpose of this study is to assess the actual food source of *Astyanax* cavefish. Stomach and intestine contents were analysed from nine fry and five adults captured during both dry and rainy seasons from the Pachón cave, which is where the most commonly studied population of *Astyanax* lives.

Methods

Specimens

Six *Astyanax mexicanus* cavefish fry and two adults were collected in Pachón cave, Tamaulipas, Mexico during the dry season (3/21/2016) and three fry and three adults during the rainy season (8/5/2016). Collecting permit # SGPA/DGVS/02438/16 from Secretaría del Medio Ambiente y Recursos Naturales, México, was issued to Patricia Ornelas García. Sample size was kept to the minimum to achieve the goals of the study. Currently the cave morph of *A. mexicanus* is in the IUCN Red List of Threatened Species. A larger sample size was not required as ranges of prey consumed between fry and adults did not overlap, variability was comparatively low, and statistical significance (Mann-Whitney U test) could be achieved with a small sample size that pose no threat to its for conservation.

Specimens were sacrificed in the field immediately after collection and deposited in 100% ethanol to prevent further digestion of gut contents. Photographs were taken on the field with a Canon EOS100 camera.

Gut contents

Specimens fixed in the field were brought to the laboratory and dissected with the aid of a Motic-K series stereomicroscope, scalpel, scissors, and dissection needles. Stomach and intestines were dissected and analysed separately to differentiate recently ingested food from the older, more degraded and digested food. All gut contents were examined in detail with 4× to 50× magnification on a Motic-K series stereomicroscope and separated into: 1) complete or partial organisms whose identity could be established at least to the taxonomic level of class, 2) fragments of organisms whose taxonomic identity were unclear, and 3) glop substance or "gunk" without identifiable structures. Percentage of the composition of each class of gut content was then estimated by distributing all contents on a petri dish to create a compact, uniform, flat layer of food content in which each class of content was separated. Then the area and volume covered by each group would be compared to obtain a percentage estimate of total volume of food content within each item. To obtain images of the gut contents, multiple pictures focused in different depth planes were photographed under an optic microscope. The Zerene Stacker focus stacking software was then used to obtain single images where the entire subject is in focus.

Results

Discovery of juvenile fish in the Pachón cave

The main pool containing *Astyanax* cavefish is at the south-eastern end of the Pachón cave. About ten meters before it, there is a small and narrow passage on the right hand side which, during the rainy season, may have a small stream that flows into the main pool. During the dry season, the side gallery only has a couple of isolated ~2 m long pools (Fig. 1). These small pools are where the fry specimens were collected, sacrificed immediately after collection and deposited in 100% ethanol so as to prevent further digestion of gut contents. At both dates of collection (March and August 2016), the estimated density of adult and fry fish in these pools was one fish for every four to five litres. Fish in these smaller pools were at much higher densities (at least 20×, but the exact number was not quantified in the field) than in the main pool. This was probably due to being stranded and concentrated as the dry season reduced the area of these pools. In the main pool, adult fish were present, but no fry were evident.

Fry were very abundant in March, but scarce in August, when only five fry were counted. The total lengths of the fry collected in March were 1.5, 1.7, 1.7, 1.8, 1.9 and 2 cm (Fig. 2A and C) and the samples collected in August were 1.0, 1.7 and 1.7 cm. The captured Pachón fry had no scales, transparent jaws and bodies, larval-like jaws and body shapes, and incompletely regressed eyes (Figs 2A and 3A). They had 6 to 9 rays on their dorsal fins (definitive number: 11), 18 to 21 rays on their anal fins (definitive number: 20–21), and their adipose fin showed non mature aspect and size

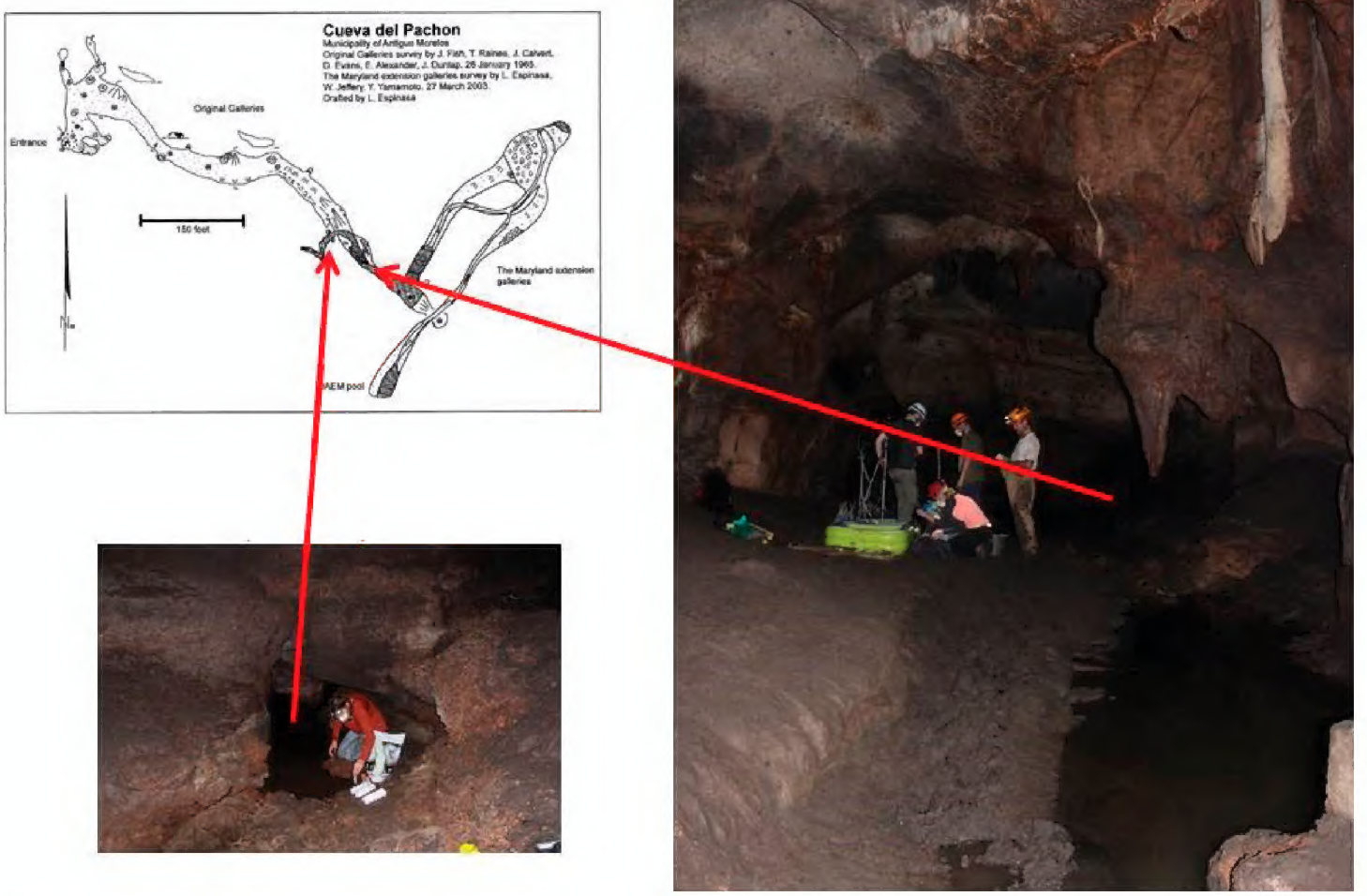
Cueva del Pachon
Municipality of Antiguo Morelos
Original Galleries survey by J. Fish, T. Raines, J. Calvert, D. Evans, E. Alexander, J. Dunlap, 26 January 1965.
The Maryland extension galleries survey by L. Espinasa, W. Jeffery, Y. Yamamoto, 27 March 2003.
Drafted by L. Espinasa
Original Galleries
Entrance
150 feet
The Maryland extension galleries
PEM pool

Figure 1. Pachón cave map (from NNS News, September 2003, p255.). Adult fish were found in the main pool (Right arrow). Adult fish and post-larval fish were found in small pools in a side gallery (Left arrow).

(see Hinaux et al. 2011). These morphological criteria correspond to post-larval stages. Fry of this size that were raised in the laboratory and fed under optimum conditions would be about 2.5 months old (Fig. 2B). However, in natural conditions, fish growth is probably much slower and we estimate that the captured specimens must be older than 2.5 months.

The pools were also inhabited by the Mysid shrimp, *Speleomysis quinterensis* Villalobos, 1951 (Crustacea, Mysidacea) and by the Isopod, *Speocirolana pelaezi* Bolivar, 1950 (Crustacea, Isopoda), respectively 3 cm and 1 cm long (Fig. 2D and E). Observations on-site in March also detected the presence of what were, presumably, copepods or water fleas. When the bottom of the substrate was gently disturbed with a finger, an average of one of these microscopic crustaceans every 4 cm2 was seen fleeing.

Stomach contents of post-larval fish in the Pachón cave

All fry appeared to be well-fed and had an abundance of food items in their stomachs and intestines (Fig. 3C). On average, fry had 17.9 readily identified food items in their guts, with a minimum of 5 and a maximum of 51 (Tables 1 and 2). Most identified

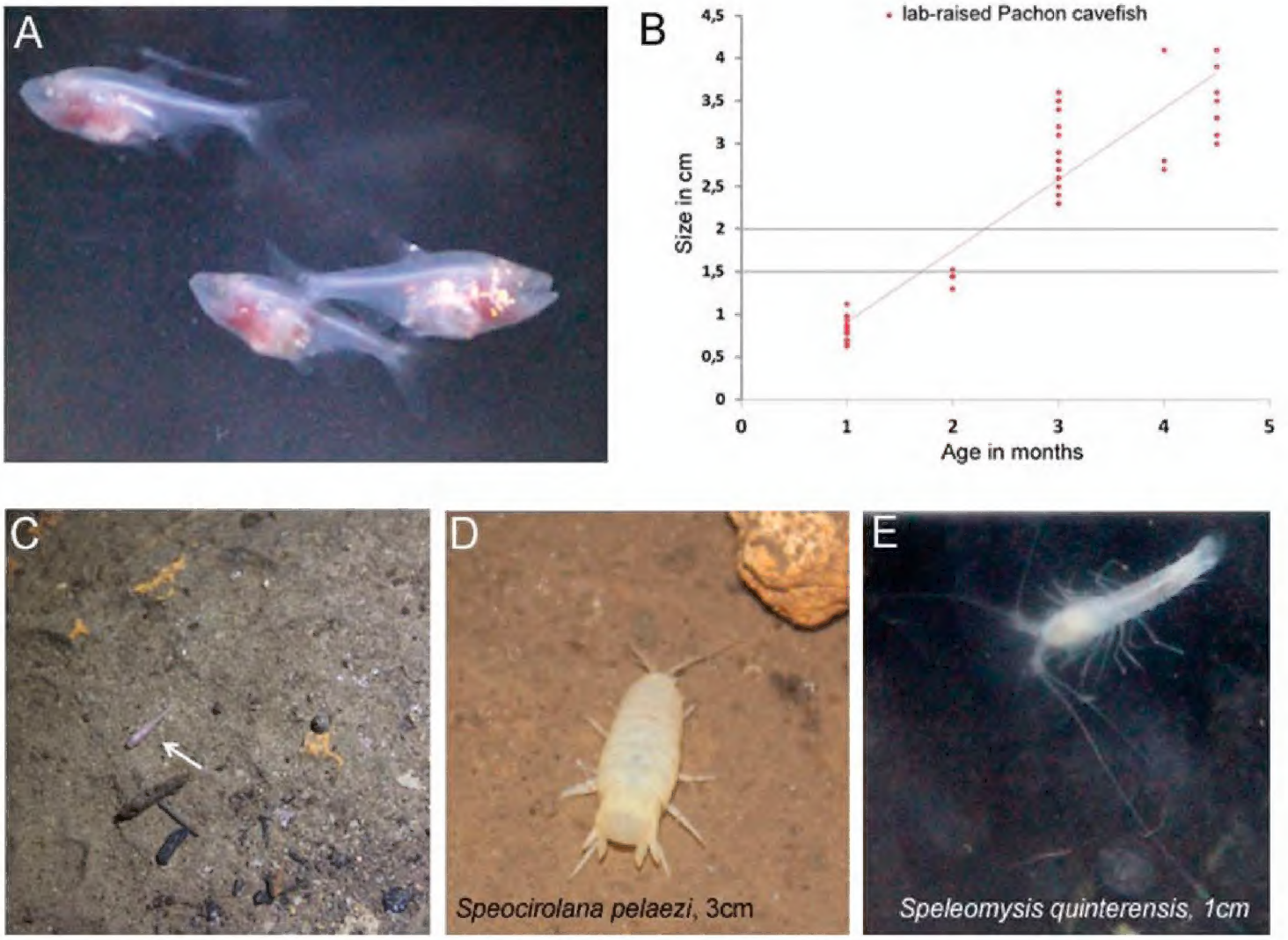

Figure 2. Size and estimated age of the fry captured in the Pachón cave. **A** Live Pachón fry photographed in a small fish aquarium, in the cave **B** Size/age relationship for lab-raised Pachón individuals with a linear regression curve. Data were collected in Rétaux's lab from larvae, post-larvae and juvenile grown as described in Elipot et al. (2014), and which were fed twice a day with live *Artemia, ad libitum* **C** Photograph of a specimen swimming in the natural pool. Note the muddy/sandy substrate and the low water level **D, E** Photographs of live arthropod specimens cohabiting with *Astyanax* fry.

Table 1. Items of food found in the guts of *Astyanax* fry (1.5–2 cm) collected in the dry season.

Food item	Fry # 1	Fry # 2	Fry # 3	Fry # 4	Fry # 5	Fry # 6	Average
Cladocera Water fleas (A–C)	7	3	14	8	17	34	**13.83**
Harpacticoida copepod (D)	3	7	11	1	4	11	**6.16**
Ostracod (G)	0	4	0	0	2	4	**5.5**
Unidentified arthropods (I)	0	0	1	1	1	0	**0.5**
Copepod (E)	0	1	0	0	1	0	**.33**
Copepod (F)	0	0	1	0	0	0	**0.16**
Isopod (H)	0	0	0	0	0	1	**0.16**
Nematode	0	0	0	0	0	1	**0.16**

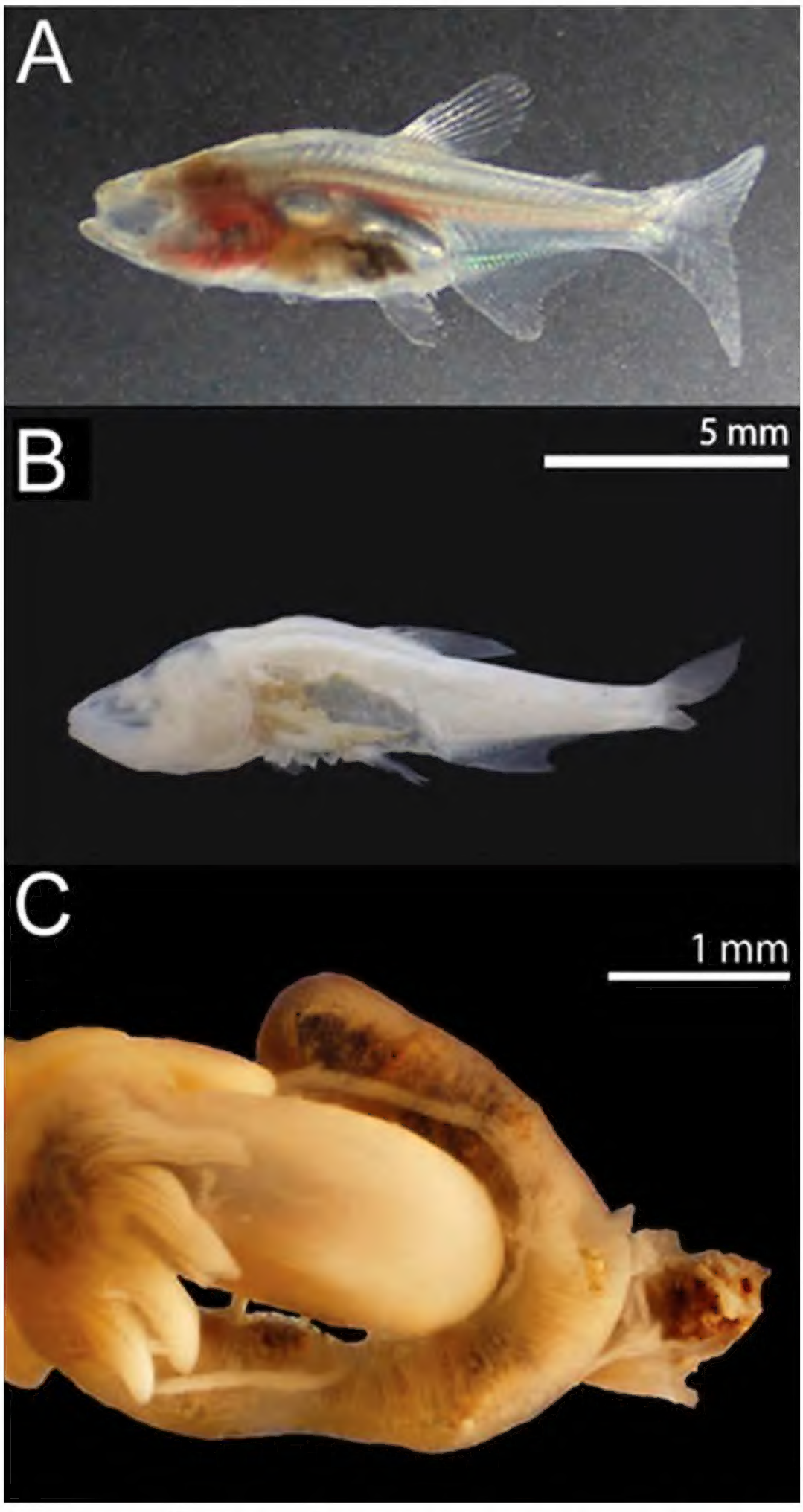

Figure 3. Digestive system of an *Astyanax* fry. **A** A live specimen photographed in the Pachón cave. Note the healthy-looking appearance of this juvenile, the two parts of the inflated swim bladder, the almost completely degenerated eye, and the digestive system filled with food. Scale bar as in B. **B** Body, with the digestive system exposed **C** Stomach and intestine. Notice that the food content can be seen through the translucent walls. All fish studied were well fed and their guts were full of food.

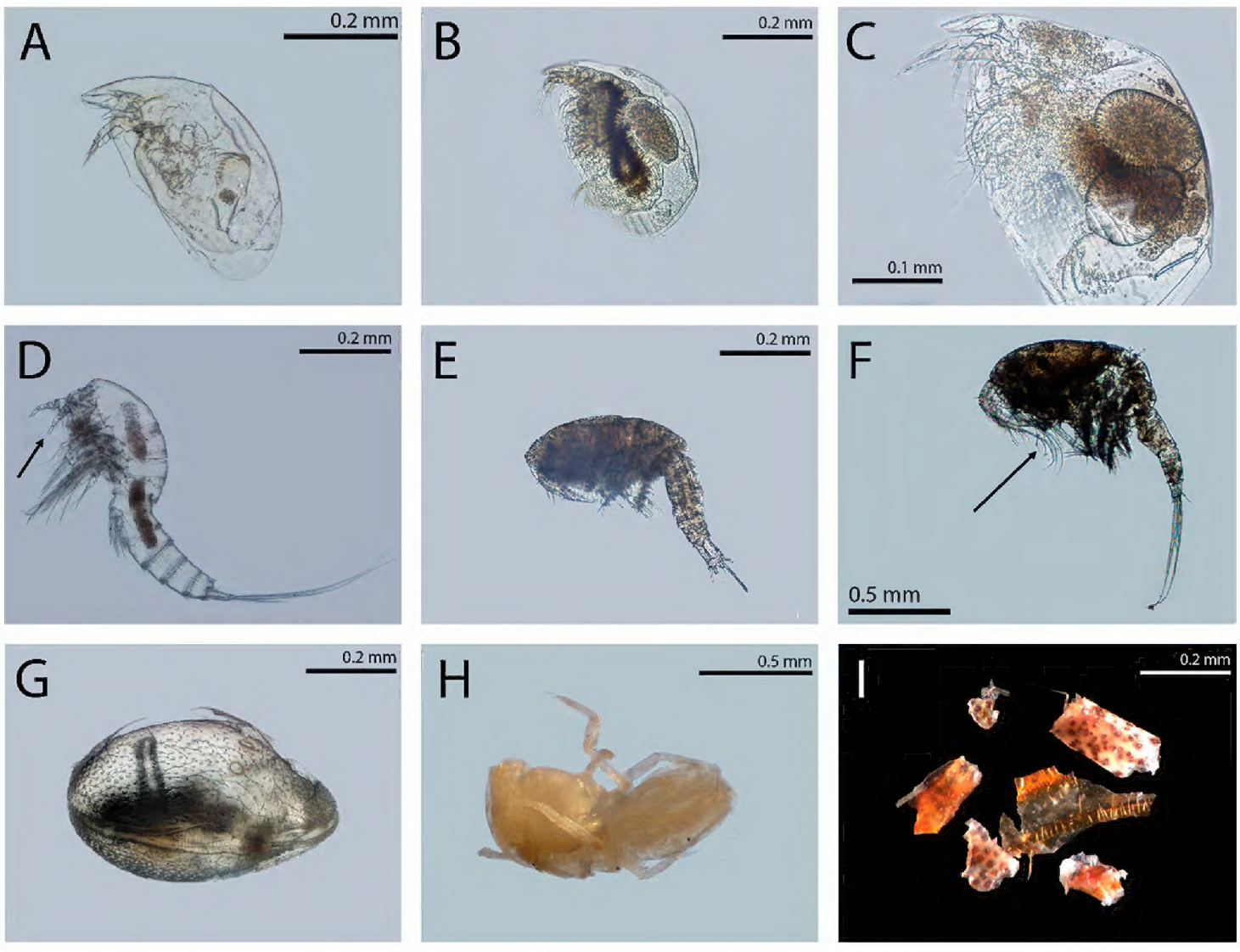

Figure 4. Gut contents of Pachón cave fry. **A–C** Cladocera Water fleas. This species constituted by number the most encountered prey. On average, fry had in their guts 9.3 individuals of this species **D** Harpacticoida copepod. Arrow highlights the short antennae diagnostic of class Harpacticoida. This species constituted by number the second most encountered prey. On average, fry had in their guts 4.7 individuals of this species **E–F** Copepods. Arrow highlights the long antennae diagnostic of non-harpacticoida copepods **G** Ostracod. This and possibly two more species of ostracods were in their guts **H** Isopod. While only one specimen was eaten, due to its large size it constitutes a large stomach content by volume **I** Sclerites of arthropods, possibly of insects. Contrary to all of the above, they have pigment, suggesting that some may be surface insects. Some may be a by-product of eating guano from insectivorous bats.

Table 2. Items of food found in the guts of *Astyanax* fry (1–1.7 cm) collected in the rainy season.

Food item	Fry # 7	Fry # 8	Fry # 9	Average
Cladocera Water fleas (A-C)	0	1	0	**0.33**
Harpacticoida copepod (D)	0	5	0	**1.66**
Ostracod (G)	0	0	1	**0.33**
Unidentified arthropods (I)	1	1	2	**1.33**
Copepod (E)	9	1	0	**3.33**
Copepod (F)	0	0	2	**0.66**
Isopod (H)	0	0	0	**0**
Nematode	0	0	0	**0**

items belonged to arthropods, and in particular, to microscopic crustaceans. Gut items belonged to water fleas Cladocera (Fig. 4A–C), at least three species of copepods (Fig. 4D–F) with at least one species belonging to the Harpacticoida order (Fig. 4D), at least two species of ostracods (Fig. 4G), one isopod (Fig. 4H), one nematode, and several fragments of chitin most likely originating from the exoskeleton of insects (Fig. 4I).

By far the most common food item was the water flea (Fig. 4A–C). On average, each fry had 9.3 individuals of this species in their stomach and intestine, with one fry containing at least 34. They constitute 52.1% of the identifiable items ingested. This was followed by the Harpacticoida copepods (Fig. 4D), of which each fry had an average of 4.7 specimens in their gut, constituting 26.1% of the identified food items. When crustaceans were found in the stomach instead of the intestine, they were complete with well-preserved internal organs. This suggests that they had been ingested alive rather than as partially decomposed cadavers, thus supporting that the fry had hunted and swallowed live prey. When excluding the possibly parasitic nematode and the seven stools with unidentified arthropods sclerites that may have been ingested by scavenging cadavers or as part of the contents of bat guano, 95% of the gut content items that were identified were most likely hunted as live prey.

By volume of stomach contents (excluding intestine contents where prey may have already been digested into gunk), the identifiable crustaceans constituted 60.6% of the total (Non-harpacticoida copepods 26.6%, Isopods 15%%, water fleas and ostracods 9.5%, and the Harpacticoida copepods 9.5%). Another 29.2% consisted of unidentifiable fragments of arthropods, and the remaining 10.2% was gunk (Table 3). When the volume of items was analyzed from the intestine, where digestion and degradation had further progressed, the proportional total volume of gunk increased to 26.1% at the expense of the other items.

Stomach contents of adult fish in the Pachón cave

Six adult fish were captured in the main pool of the Pachón cave. Two adults (standard length: 3.6 and 4.1cm) were collected in March in addition to three adults (3.7, 4.1 and 4.6 cm) in August. Gut contents of adult fish were drastically different from post-larval fish. At least in these five specimens, we did not find body parts that suggest predation of either the microscopic crustaceans, or the macroscopic Mysid shrimp, *Speleomysis quinterensis* or the isopod, *Speocirolana pelaezi* that cohabitate with *Astyanax* in Pachón cave. There were only two items in the guts of two specimens that suggest predation of a live prey; a single fly and a single beetle (Fig. 5E–F). The bodies of these arthropods were entire and thus were likely eaten while still alive. By volume of stomach and intestine contents, these prey constituted only 15% and 6% respectively. The rest of the gut contents in these five adult individuals were consistent with a diet primarily comprised of partially decomposed material, guano, or detritus from the mud (Fig. 5 and Table 4). The most abundant stomach content was gunk primarily composed of arthropod sclerites (20%), which could be derived from the guano of

Table 3. Stomach and intestine contents by volume in *Astyanax* fry (1–2 cm).

	Unidentified arthropods (I)	Isopod (H)	Cladocera Ostracods (A–C, G)	Harpacticoid copepod (D)	Copepod (E-F)	Gunk	Nematode
Stomach March	43.75%	22.50%	12.50%	8.75%	6.25%	6.25%	0%
Intestine March	19.25%	0%	31.25%	17.5%	0%	30.75%	1.25%
Stomach August	0%	0%	3.6%	10.9%	67.3%	18.2%	0%
Intestine August	52.2%	0%	11.0%	6.7%	13.4%	16.7%	0%

Table 4. Stomach (bold) and intestine (non-bold) contents by volume in adult *Astyanax* (3.6–4.6 cm).

Food item	Adult # 1	Adult # 2	Adult # 3	Adult # 4	Adult # 5	Average
Fly or beetle	- -	- -	- -	**75%** -	- 30%	**15%** 6%
Gunk with sclerites (A-C)	- -	- 50%	- -	- 25%	**100%** 70%	**20%** 29%
Black gunk (D)	**40%** -	- -	- 5%	- -	- -	**8%** 2%
White gunk (G)	**60%** 60%	- 50%	- 90%	- -	- -	**12%** 40%
Yellow gunk (I)	- 35%	- -	-	- -	- -	**0%** 9%
Mud	- 5%	- -	- 5%	**25%** 75%	- -	**5%** 16%

insectivorous bats. Also, in the stomach there was an estimated 13% of soil detritus (8% of black gunk and 5% of mud).

All fry and adult fish had items in their intestines. Nonetheless, it is noteworthy that while all nine fry had at least some food items in their stomach, two out of five adult fish had an empty stomach.

Discussion

Our results show that post-larval fry from Pachón cave appear to be well-fed and are efficient predators. This is evident in the guts of the nine individuals that contained an average of 17 specimens of microscopic crustaceans. Our results show that arthropods are the main source of nourishment for 1-2 cm long *Astyanax* fry in Pachón cave, with 89.8% of their stomach contents being readily identifiable arthropods. Data suggests that they are active hunters of aquatic water fleas, ostracod, copepod and isopod crustaceans, which constitute 60.6% of the total food volume found in their stomachs. Only 10.2% of the stomach content by volume belonged to the type of unidentifiable gunk in fry. It is likely

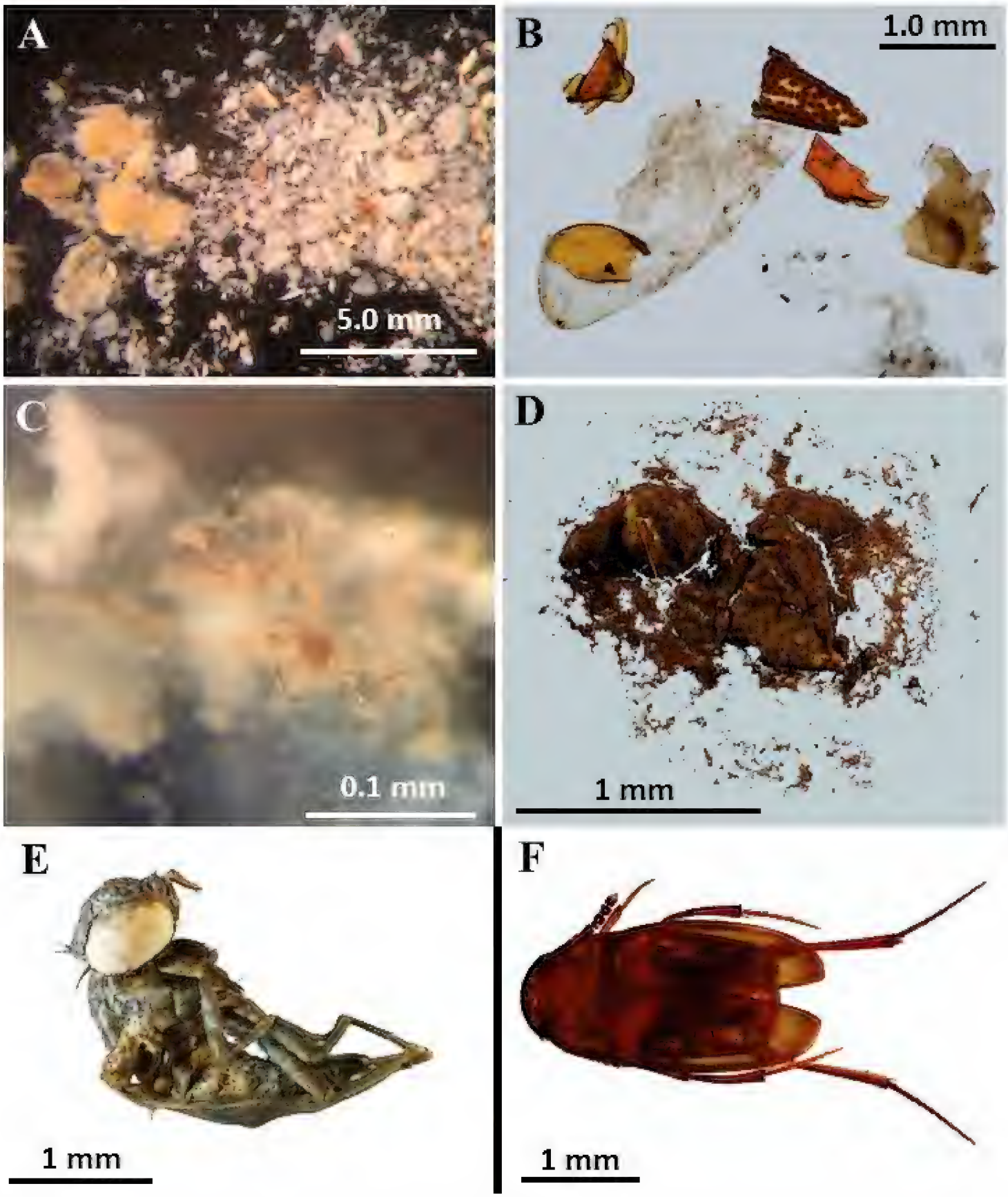

Figure 5. Gut content of adult Pachón cave fish. **A** White, orange, and black "gunk" of undetermined origin **B** Hair-like filaments in stomach contents **C** Unidentified pigmented arthropod sclerites, possibly of surface insects or by-product of eating guano. **D** Mud **E** Fly **F** Beetle.

that during this stage, for the Pachón cave population and perhaps other cave populations, arthropods constitute most of their nourishment through active predation.

While all fry specimens were well-fed with their guts containing considerable amount of contents, it appears that those specimens collected during the dry season (March) were proportionally better-fed than those collected in the rainy season

(August). The first had an average of 23 readily identified food items in their guts, while the second had an average of 7.7 readily identified items. It is also noteworthy that the gunk content found in the stomach increased from 6.2% to 18.2% in the rainy season. While intestine gunk may represent digested prey, stomach gunk is likely to be indicative of the fish eating guano, decomposed detritus, or mud. It may be that during the rainy season, fry had less live prey available for sustenance and relied on other nourishing items to supplement their consumption. An example supporting this idea is that in one fry, a pellet of stool gunk was largely comprised of insect scales, most likely from guano droppings originating from a bat that ate moths.

It may seem counterintuitive that there are more stomach contents in the fry during the dry season than the rainy season. One would expect that more food items become available during the wet season. Our experimental protocol was not designed to provide an explanation and we can only report observed results. Future studies will document ecological parameters as well as environmental conditions throughout a full year. Nonetheless, while the idea of seasonal flooding bringing items into the caves is an appealing one, one should remember that each cave is a unique case. Pachón cave does not have a stream flowing into it during the rainy season, nor does it experience flash flooding in ways similar to Rio Subterraneo, Tinaja, Sabinos or other caves. Pachón cave has a sump pool where more or less filtrated water trickles in. Water level of the pool increases or decreases, without the influx of large debris being flushed in flash floods. One of the many possibilities is that microscopic crustaceans that come from the epikarst are actually diluted during the rainy season. Only future longitudinal studies will be able to resolve this conundrum.

There is a plethora of literature on *Astyanax* cavefish discussing the adaptations that allow them to be very skilful at locating nourishment in an environment where food is often scarce (Espinasa et al. 2014; Rétaux and Elipot 2013; Soares and Niemiller 2013; Yoshizawa 2015). Our results confirm that they are indeed excellent hunters, but suggest that, at least in this cave and for this particular stage in their ontogeny, food is not that limiting.

There are very few published materials regarding what *Astyanax* cavefish actually eat in their natural environment. To our knowledge, this has been restricted to adult fish. Wilkens and Burns (1972) studied the gut content of 17 specimens from Río Subterraneo cave in the Micos area, whose total length varied from 3-10 cm. Their stomachs and intestines were filled with a brown substance, which was interpreted as guano from insectivorous bats that cover the floor in parts of this cave. Examination of this brown substance showed that it was mostly fragments of chitin originating from the exoskeleton of insects and considerable amounts of scales from wings of nocturnal Lepidoptera. Some large specimens of the Micos fish also contained partly digested fish bodies, suggesting cannibalism or carrion feeding on their conspecifics. In the five Pachón adults we examined, their gut contents were also primarily gunk. Identifiable structures were also pigmented chitin fragments originating from the exoskeleton of arthropods that inhabit the cave or as part of the guano of bats. Contrary to the post-larval fish where

they averaged 17 ingested preys, the five adult specimens did not exhibit evidence for active hunting of crustaceans, and only showed the consumption of a single fly and a single beetle, leading to an average of 0.4 prey ingested per individual.

Some authors have suggested that *Astyanax* food consists almost completely of bat guano rather than live and mobile organisms (Kasumyan and Marusov 2015). It is unlikely that bat guano is the primary food item throughout their development and across the entire range of cave *Astyanax*. Firstly, the large interconnecting underground system that makes the habitat of cave *Astyanax* (Espinasa and Espinasa 2015) is composed of thousands of kilometres of underground passages that are beyond the reach of bats. While cave biologists studying the over 30 described localities of cave *Astyanax* often encounter bats, both human and bat advancement into the cave is stopped by sumps that fish can swim through. The largest proportion of the *Astyanax* populations undoubtedly inhabits the unreachable, larger sections of the underground system beyond these sumps. Fish in these sections must find nourishment from sources other than bat droppings.

A second reason to doubt that bat guano is their single source for nourishment is based on the presence of other non-smell detecting adaptations that are fine-tuned for locating live prey, including VAB. As mentioned before, many crustaceans in the water column produce 30-40 Hz water fluctuations while swimming (Lang 1980). Most Pachón fish have VAB, while most surface fish lack it. Even in the surface fish that have VAB, the ability to "tune-in" to certain vibration frequencies is absent and they have a broad response range from 5-50 Hz. By contrast, cavefish have a significant peak at 35 Hz brought by changes in the morphology and number of superficial neuromasts within the orbit of the degenerated eye (Yoshizawa 2015). QTL analyses have shown multiple genomic loci for VAB, superficial neuromast number at the eye orbit, and eye size. Importantly, the QTL for all three traits overlap each other in two regions of the *Astyanax* genome (Yoshizawa 2015).

We do not argue that guano is not a source of food. The five adult specimens examined from Pachón cave, anecdotal comments by colleagues, and our own personal unpublished observations of gut contents from other caves suggest that many El Abra populations have gut contents composed of gunk suggestive of eating guano or detritus from the mud. *Astyanax*, like many other cave adapted organisms, is probably a generalist and an opportunist. It is likely that its source of nourishment varies greatly not only between caves, but also throughout its ontogeny. Such is the case for surface *Astyanax*. A surface population studied by Wolff et al. (2009) showed that in fish of less than 5 cm standard length, 75.8% of their diet by volume was made of animal items. In 5.1 to 7.5 cm long fish, animal content decreased to 28.6%, and when longer than 7.6 cm, its animal derived diet was reduced to 20.7% to the increase of plant items.

Here, we also found that the food regime in Pachón cave *Astyanax* varies greatly between post-larval 1-2cm long individuals and ~4cm long adults. This may occur in other *Astyanax* cave populations as well. Young fish are highly dependent on their hunting skills and their food is significantly made of nourishing sources such as micro-

scopic crustaceans. As they get older and larger, these microscopic animals become a less effective source of food. They then change their diet to more abundant but perhaps less nourishing sources like guano or mud detritus, as well as the opportunistic insect or carcass that may fall in or brought in during the rainy season. The idea that there is a change in the cavefish's diet that reduces their dependence for hunting live prey is supported by the observation that in large, mature adult cavefish (> 6 cm long), superficial neuromasts showed reduced sensitivities compared to those in smaller, younger adults (< 4 cm long), corresponding to a significantly attenuated VAB in large Pachón cavefish (Yoshizawa 2015).

Conclusions

Fry had an average of 17 (+/- 14.5 StDev; Range=3-50) prey in their guts while adults had an average of 0.4 (+/- 0.5 StDev; Range=0-1) prey, which is significantly different (P=.001). In conclusion, our results suggest that at a young stage when the yolk has been depleted and young larvae must find food for themselves, *Astyanax* cavefish's enhanced skills for prey capture become the primary means for obtaining nourishment. Close to 90% of their food items may derive from arthropods and at least 60.6% by volume are the likely product of active hunting of microscopic crustaceans. These enhanced hunting skills in fry are probably essential for the survival within the cave environment. These skills may be modulated by the enhancement of superficial neuromast activity. Other options may be possible, such as the enhancement of mechanosensors, chemical sensors, or performance of the brain, to name a few. *Astyanax* diet changes with age, probably as microscopic crustaceans are no longer adequate for larger specimens. Adult cavefish probably feed on a variety of stationary and moving items in cave pools that may progressively rely less on VAB. Stationary objects located at the bottom of cave pools, such as particles of detritus, washed-in cadavers, or bat guano, could be more efficiently detected using olfactory cues and an enlarged olfactory pit.

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